DOCKET NO.: ISIS0001-100 (CORE0027US) **PATENT**

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Please add new claims 83 and 84.

Please amend claims 53-58, 60-71, 75 and 79-82 as follows:

1. **(Original)** A method of identifying a multifunctional oligomeric compound to modulate expression of RNA comprising:

(a) contacting a target RNA with one or more double-stranded oligomeric compounds hybridizable to one or more target regions of said RNA and identifying double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%;

(b) contacting the target RNA with an antisense strand of said modulating double-stranded oligomeric compound and determining whether the antisense strand inhibits target RNA levels by at least 50%; and

(c) identifying said inhibiting antisense strand and said inhibiting double-stranded oligomeric compound as multifunctional oligomeric compounds.

2. **(Original)** A multifunctional oligomeric compound identified according to claim 1.

3. **(Original)** A method of claim 1 wherein the multifunctional oligomeric compound inhibits target RNA levels by at least 80%.

4. **(Original)** The method of claim 1 wherein the target region is identified by a single-stranded oligomeric gene walk across the target RNA.

5. **(Original)** The method of claim 1 wherein the target region is identified by secondary structure analysis of the target RNA.

6. **(Original)** The method of claim 1 wherein said target region is at least a portion of an induced gene.

7. **(Original)** The method of claim 6 wherein the induced gene is CD54.

8. **(Original)** The method of claim 1 wherein said target region is at least a portion of a constitutive gene.

9. **(Original)** The method of claim 1 wherein said target region is localized to the 3'UTR, the 5'UTR, an intron:exon boundary, an exon:exon boundary, a start region or a coding region of the RNA.

10. **(Original)** The method of claim 1 wherein said target region is localized to the 3'UTR.

11. **(Original)** The method of claim 1 wherein said target region is localized to the 5'UTR.

12. **(Original)** The method of claim 1 wherein said target region is localized to an intronic portion of a gene.

13. **(Original)** The method of claim 1 wherein said target region is localized to an exon.

14. **(Original)** The method of claim 1 wherein said target region is localized to an intron/exon boundary.

15. **(Original)** The method of claim 1 wherein said target regions overlaps the intron/exon boundary with 5-10 nucleotides on either side of the boundary.

16. **(Original)** A method for optimizing target region selection for modulation of RNA expression comprising:

(a) contacting one or more double-stranded oligomeric compounds with one or more regions of a target RNA and identifying target regions which, when contacted with the one or more double-stranded oligomeric compounds, result in inhibition of target RNA levels of at least 50%;

(b) contacting one or more single-stranded oligomeric compounds with said inhibited target regions and identifying regions which, when contacted with the one or more single-stranded oligomeric compounds, result in inhibition of target RNA levels of at least 50%;

(c) identifying regions modulated by at least one double-stranded oligomeric compound and at least one single-stranded oligomeric compound as optimized target regions.

17. **(Original)** The method of claim 16 wherein target RNA levels are inhibited by at least 80% by single-stranded oligomeric compounds and double-stranded oligomeric compounds.

18. **(Original)** The method of claim 1 wherein the oligomeric compound is an antisense oligonucleotide.

19. **(Original)** The method of claim 1 wherein the oligomeric compound has at least one modification of the base, sugar or internucleoside linkage.

20. **(Original)** The method of claim 1 wherein the oligomeric compound has a modification at the 2' position of at least one sugar.

21. **(Original)** The method of claim 1 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside.

22. **(Original)** The method of claim 1 wherein said oligomeric compound is from about 12 to about 50 nucleotides in length.

23. **(Original)** The method of claim 1 wherein said oligomeric compound is from about 18 to about 25 nucleotides in length.

24. **(Original)** The method of claim 1 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

25. **(Original)** The method of claim 1 wherein the oligomeric compound is RNA.

26. **(Original)** The method of claim 1 wherein the oligomeric compound is a siRNA

27. **(Original)** The method of claim 1 wherein said hybridization is under moderate or high stringency conditions.

28. **(Original)** The method of claim 1 wherein the oligomeric compound is a potent modulator of the target RNA.

29. **(Original)** The method of claim 1 wherein the oligomeric compound is a gapmer.

30. **(Original)** The method of claim 1 wherein the oligomeric compound comprises at least six consecutive nucleosides with 2' modifications.

31. **(Original)** The method of claim 1 wherein the oligomeric compound is a hemimer.

32. **(Original)** The method of claim 1 wherein the oligomeric compound comprises at least one phosphorothioate linkage.

33. **(Original)** The method of claim 1 wherein the oligomeric compound is a chimeric compound.

34. **(Original)** The method of claim 1 wherein the oligomeric compound comprises one or more chimeric regions.

35. **(Original)** The method of claim 1 wherein the target RNA is preselected.

36. **(Original)** A method of modulating RNA expression comprising contacting target regions optimized according to claim 16 with two or more oligomeric compounds.

37. **(Original)** A method of optimizing modulation of RNA comprising contacting a target RNA with at least two oligomeric compounds hybridizable to a target region of said target RNA wherein at least two oligomeric compounds each inhibit RNA levels by at least 50% when tested individually.

38. **(Original)** A method of optimizing target regions of RNA comprising:

(a) contacting a target RNA comprising a target region with a plurality of oligomeric compounds hybridizable with said target region; and,

(b) identifying target regions as optimized when two or more of said oligomeric compounds inhibit target RNA levels by at least 50%.

39. **(Original)** The method of claim 38 wherein the oligomeric compound comprises at least one double-stranded region.

40. **(Original)** The method of claim 38 wherein target regions are identified as optimized when two or more of said oligomeric compounds inhibit target RNA levels by at least 80%.

41. **(Original)** A method of selecting a target region of a gene comprising:

(a) contacting a target RNA comprising at least one target region with a plurality of oligomeric compounds hybridizable with said at least one target region, wherein said oligomeric compounds comprise at least one siRNA oligomeric compound and at least one ASO oligomeric compound;

(b) identifying siRNA and ASO oligomeric compounds which inhibit RNA levels by at least 60% for each of said at least one target regions; and

(c) selecting target regions when there is a significant association between inhibiting siRNA oligomeric compounds and ASO oligomeric compounds for the target region.

42. **(Original)** The method of claim 41 wherein at least one of said oligomeric compounds comprises at least one double-stranded region.

43. **(Original)** A method of claim 41 wherein (c) is performed using a ROC analysis.

44. **(Original)** A method of claim 43 wherein the ROC analysis yields an area under the curve of at least 0.6 .

45. **(Original)** A method of claim 43 wherein the ROC analysis yields an area under the curve of at least 0.8 .

46. **(Original)** A target region of a gene selected according to the method of claim 41.

47. **(Original)** A method of selecting an optimized single-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more double-stranded oligomeric compounds;

(b) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) selecting the strand of the double-stranded oligomeric compound that hybridizes to the target RNA as the optimized single-stranded oligomeric compound.

48. **(Original)** The method of claim 47 wherein target RNA levels are inhibited by at least 80%.

49. **(Original)** A method of selecting an optimized double-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more single-stranded oligomeric compounds;

(b) identifying one or more single-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) hybridizing a complementary single-stranded oligomeric compound to said single-stranded oligomeric compound, thereby yielding an optimized double-stranded oligomeric compound.

50. **(Original)** A method of selecting a single-stranded oligomeric compound comprising;

(a) contacting a target RNA with one or more double-stranded oligomeric compounds;

(b) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) selecting the strand of the identified double-stranded oligomeric compound which is complementary to the target RNA as the selected single-stranded oligomeric compound.

51. **(Original)** A method of selecting a double-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more single-stranded oligomeric compounds;

(b) identifying one or more single-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) hybridizing a complementary single-stranded oligomeric compound to said identified single-stranded oligomeric compound, yielding a double-stranded oligomeric compound as the selected double-stranded oligomeric compound.

52. **(Original)** A method of identifying one or more optimized double-stranded oligomeric compounds comprising:

(a) cloning one or more target regions from a target RNA into a vector/plasmid construct;

(b) transfecting said vector/plasmid into a cell;

(c) contacting a cell transfected with said vector/plasmid with one or more double-stranded oligomeric compounds, said compounds having one strand hybridizable to said target region; and,

(d) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%.

53. **(Currently amended)** The ~~An~~ oligomeric compound ~~, 8-80 nucleobases in length, targeted to a target RNA, wherein said oligomeric compound specifically hybridizes said target RNA and~~ of claim 83 wherein said oligomeric compound inhibits RNA levels by at least 50% in both single-stranded and double-stranded forms.

54. **(Currently amended)** The oligomeric compound of claim ~~53~~ 83 wherein the oligomeric compound comprises one or more hairpin regions.

55. **(Currently amended)** The oligomeric compound of claim ~~53~~ 83 wherein RNA levels are measured in A549 cells.

56. **(Currently amended)** The ~~An~~ oligomeric compound ~~, 8-80 nucleobases in length targeted to a target RNA, wherein said oligomeric compound has at least 80% sequence homology to the complement of said target RNA and~~ of claim 84 wherein said oligomeric compound inhibits RNA levels by at least 60% in both single-stranded and double-stranded forms.

57. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein the sequence homology is at least 90%.

58. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein the oligomeric compound has at least 2 mismatches as compared to the complement of the target RNA.

59. **(Original)** The oligomeric compound of claim 58 wherein the mismatches are internal or external base mismatches.

60. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein no more than two of the four 3'-most nucleotides of the oligomeric compound are mismatches.

61. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound has an IC50 no greater than 100nM.

62. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound has an IC50 no greater than 10nM.

63. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound is targeted to the 3'UTR, the 5'UTR, an intron:exon boundary, an exon:exon boundary, a start region or a coding region of the RNA.

64. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound is targeted to the 3'UTR.

65. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound is targeted to the 5'UTR.

66. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound is targeted to an intronic portion of the RNA.

67. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound is targeted to an exon.

68. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound is targeted to an intron/exon boundary.

69. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound has alternating linkages.

70. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein the oligomeric compound has alternating modifications.

71. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein every second nucleotide in the antisense strand of the double stranded oligomeric compound is modified.

72. **(Original)** The oligomeric compound of claim 71 wherein the first modified nucleotide is the 5'-most nucleotide of the oligomeric compound.

73. **(Original)** The oligomeric compound of claim 71 wherein the modifications are 2' modifications.

74. **(Original)** The oligomeric compound of claim 71 wherein the modifications are one or more of 2'-O alkyl, 2'-O-methoxyethyl, 2'-methoxyethoxy, 2'-dimethylaminooxyethoxy, 2'-

dimethylaminoethoxyethoxy, 2'-methoxy, 2'-aminopropoxy, 2'-allyl, 2'-O-allyl (2'-O-CH_2-CH=CH_2), or 2'-fluoro.

75. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound comprises:

a first segment;

a second segment; and,

a third segment comprising three or four nucleobases, said third portion located between said first and second segments;

wherein said first and second segments each have at least one modified nucleobase.

76. **(Original)** The oligomeric compound of claim 75 wherein said third segment has no modified nucleobases.

77. **(Original)** The oligomeric compound of claim 75 wherein said first and second segments each comprise at least one modified linkage/modification.

78. **(Original)** The oligomeric compound of claim 77 wherein said third segment has no modified linkages or modifications.

79. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound hybridizes to at least a portion of the 3' UTR of said target RNA.

80. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

81. **(Currently amended)** The oligomeric compound of claim ~~56~~ 84 wherein said oligomeric compound comprises at least seven 2'-O-methyl substitutions at the 3'-terminus of the oligomeric compound.

82. **(Currently amended)** An oligomeric compound of claim ~~53~~ 83 wherein the oligomeric compound has at least six mismatches as compared to the complement of the target RNA.

83. **(New)** An oligomeric compound, 8-80 nucleobases in length, targeted to a target RNA, wherein said oligomeric compound specifically hybridizes said target RNA and wherein said oligomeric compound inhibits RNA levels by at least 50%.

84. **(New)** An oligomeric compound, 8-80 nucleobases in length targeted to a target RNA, wherein said oligomeric compound has at least 80% sequence homology to the complement of said target RNA and wherein said oligomeric compound inhibits RNA levels by at least 60%.